PROJECT NUMBER: 1904
PROJECT TITLE: Tobacco Physiology and Biochemistry
PROJECT LEADER: H. Y. Nakatani
PERIOD COVERED: October, 1987

I. LOW NICOTINE STUDY

A. Objective: To investigate the biochemistry of the nicotine biosynthetic pathway at putrescine N-methyltransferase (PMT) and N-methylputrescine oxidase (MPO) and specifically to isolate PMT from tobacco root extracts.

B. Status: The roots from the hydroponically-grown, Burley 21 (Bu21) tobacco plants of group six have been harvested (1,4). Three extracts from group six have been processed to obtain the 40-65% ammonium sulfate fraction (1,4). The remaining extracts from group five were fractionated by the 3 column purification series cited in last month's summary. The PMT-active fractions from the phenyl-Sepharose columns were pooled and concentrated with 65 % (saturation) ammonium sulfate. A fourth (DEAE-Sepharose) chromatographic step was also examined, with the pooled PMT-active samples from the above 3 column scheme (2,3). One of the most PMT-active fractions from this last DEAE-Sepharose column (concentrated against PEG) was examined by SDS-PAGE and 5-7 bands were visualized by Coomassie Blue staining (about 12 bands were observed by silver staining) (5). A major band was observed in the 45-60 kDa molecular weight region (the reported molecular weight for PMT is 60,000). Samples surrounding the most PMT-active fraction from the DEAE-Sepharose column were also concentrated and examined by SDS-PAGE (5). Only 1-3 bands, dependent upon the sample, were evident by Coomassie Blue staining for those fractions from this latter column. Upon silver staining the total number of bands that were detected was increased; however, only 2-3 major bands were observed.

A larger gel filtration column (Sephadex G-100, 1 X 40 cm) has been prepared and is being calibrated to determine the molecular weight characteristics of PMT (6). The separation volume for Blue dextran (mw > 2 million, for void volume determination) and cytochrome c (about 12,500 mw) was increased from about 7.5 mL for the previous (1 X 20 cm) column to about 14.5 mL for this column. This increased separation volume should be of value for the examination of the molecular weight and gel filtration characteristics of PMT. PMT-active fractions which were obtained from the FPLC Mono Q (anion exchange) column were re-applied to the Mono Q column after buffer exchange (4). Two PMT-active peaks were obtained at 30 mM and 200 mM NaCl. The PMT-active fractions from the 200 mM NaCl peak fractions from two runs were pooled, buffer exchanged and re-applied to the Mono Q column. Several peaks were obtained through the application of a modified gradient elution. Weak PMT-activity was observed at an elution profile other than 200 mM NaCl. A literature search by Pharmacia upon our request indicated that heterogeneity has been evident for the Mono Q column, i.e., more iso-electric forms observed from Mono Q than by isoelectric focusing. Our results and the literature information indicate

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considerable development is required before the Mono Q column can be used routinely, and it has been discontinued at this point in our investigations.

C. **Plans**: The harvest of the 6th group of hydroponically-grown tobacco roots will be initiated. The three column purification scheme will be used to produce a continuous amount of PMT-active protein for further work to purify PMT. The DEAE-Sepharose column will also be implemented in the purification scheme. The new gel filtration column will be used to examine the molecular weight and gel filtration characteristics of PMT. Work will also continue to produce enough sample for antibody production and screening. Binding studies with protein-A agarose beads, PMT containing extracts, and PNMT antibody will continue.

D. **References**:

1. Dunn, R.L. PM Notebook No. 7899.

2. Malik, V. S. PM Notebook No. 8402.

3. Nakatani, H. Y. PM Notebook No. 8384.

4. Sherwood, K. R. PM Notebook No. 8216.

5. Sykes, A. PM Notebook No. 8526.

6. Yu, T. PM Notebook No. 8381.

II. OTHER PROJECT ACTIVITIES:

A. TCRC presentations were given by Ved Malik, Elizabeth Mooz, Karen Rapp Sherwood and Herb Nakatani at the 41st TCRC held in Greensboro, NC (Oct. 3-7, 1987). Reviews of assigned papers were written and presented to Carol Lunsford.

B. Bruce Davies and Herb Nakatani visited with officials of Hazelton Biotechnologies Company (Vienna, Va) on October 9, 1987, to discuss immunization schedules and development of monoclonal antibodies for our studies.

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